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medium;

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5 (f) washing the IGF-I-loaded hydrophobic interaction chromatography matrix with a hydrophobic interaction wash buffer having an ionic strength sufficiently low to remove most of the non-authentic IGF-I, but not so low as to remove a significant proportion of the authentic IGF-I from the hydrophobic interaction chromatography matrix;

10 (g) eluting the adsorbed IGF-I from said hydrophobic interaction chromatography matrix by contacting said matrix with a hydrophobic interaction elution buffer, which has a sufficiently elevated pH, or sufficiently low ionic strength, to cause displacement of substantially all of the adsorbed authentic IGF-I from said matrix;

15 (h) contacting the eluate from step (g) with a sufficient quantity of a second cation exchange matrix under conditions allowing adsorption of at least about 95% of the IGF-I from the eluate;

20 (i) washing the IGF-I-loaded second cation exchange matrix with a cation exchange wash buffer having a sufficiently high ionic strength, or sufficiently high pH, to remove a significant proportion of non-authentic IGF-I, but not so high as to remove a significant proportion of authentic IGF-I;

25 (j) eluting the adsorbed IGF-I from said second cation exchange matrix by contacting said matrix with a second cation exchange elution buffer, which has a sufficiently high ionic strength, or sufficiently high pH, to displace substantially all of the adsorbed authentic IGF-I from said matrix;

30 (k) contacting the eluate from step (j), in an aqueous buffer, with a suitable quantity of a reverse phase chromatography matrix under conditions allowing adsorption of at least about 95% of the IGF-I from the eluate;

(1) washing the IGF-I-loaded reverse phase chromatography matrix with an aqueous/organic reverse phase wash buffer having an organic solvent concentration sufficiently high to remove a substantial proportion of non-authentic IGF-I, but not so high as to remove a significant proportion of authentic IGF-I;

(m) eluting the adsorbed IGF-I from said reverse phase chromatography matrix with an aqueous/organic buffer having an organic solvent concentration high enough to remove substantially all of the authentic IGF-I without removing a significant proportion of multimeric forms of IGF-I.

2. The process of claim 1, wherein the non-authentic IGF-I removed by the washing in step (f) is reprocessed at least once through steps (d) to (g), inclusive, prior to initiation of step (h).

3. The process of claim 1, wherein said IGF-I is human recombinant IGF-I.

4. The process of claim 3, wherein said human recombinant IGF-I is produced in transformed yeast cells.

5. The process of claim 4, wherein said transformed yeast cells are of the species *Pichia pastoris*.

6. The process of claim 5, wherein said species *Pichia pastoris* is of the strain GS115.

7. The process of claim 4, wherein said IGF-I is secreted into the yeast growth medium.

8. The process of claim 1, part (a), wherein said first cation exchange matrix is sulfonylpropylated and in a chromatography column.

5 9. The process of claim 1, part (b), wherein said washing consists essentially of applying to said first cation exchange matrix approximately 3.0 column volumes of 0.02 M acetic acid, followed by approximately 3.0 column volumes of 0.05 M sodium acetate (pH 5.5).

10 10. The process of claim 1, part (c), wherein said eluting consists essentially of applying to said first cation exchange matrix between 3 and 10 column volumes of 0.05 M sodium acetate (pH 5.5) and 0.4 M sodium chloride.

15 11. The process of claim 1, part (d) wherein said unfolding/refolding buffer consists essentially of: between about 1.5 and 3.0 M urea; between about 1.0 and 3.0 M sodium chloride; between about 5% and 20% (v/v) ethanol; between about 1 mM and 15 mM sodium borate; and between about 0.005 mM and 10.0 mM dithiothreitol; and has a pH between about 8.5 and 10.0.

20 12. The process of claim 1, part (d) wherein said unfolding/refolding buffer consists essentially of 2 M urea, 1.5 mM sodium chloride, 15% ethanol, 5 mM sodium borate and 0.2 mM DTT and has a pH between about 9.0 and 9.5.

25 13. The process of claim 1, part (e), wherein said hydrophobic interaction chromatography matrix is a butyl-substituted, polymethacrylate matrix.

14. The process of claim 1, part (f), wherein said washing consists essentially of applying to said hydrophobic interaction chromatography matrix approximately three column volumes of 0.2 M acetic acid, containing 0.5 M sodium chloride; followed by approximately ten column volumes of 0.2 M acetic acid, containing 0.15 to 0.25 M sodium chloride.

15. The process of claim 1, part (g), wherein said eluting consists essentially of applying to said hydrophobic interaction chromatography matrix approximately four column volumes of 0.2 M acetic acid, containing 0 to 0.02 M sodium chloride, and having a pH of approximately 3.0.

16. The process of claim 1, part (h), wherein said second cation exchange matrix is sulfylpropylated and in a chromatography column.

17. The process of claim 1, part (i), wherein said washing consists essentially of applying to said second cation exchange matrix approximately seven to ten column volumes of 0.05 M sodium acetate buffer, containing 0.1 M NaCl, and having a pH of approximately 5.5.

18. The process of claim 1, part (j), wherein said second cation exchange elution buffer consists essentially of approximately seven column volumes of 0.05 M sodium acetate, 0.4 M sodium chloride, having a pH of approximately 5.5.

19. The process of claim 1, part (k), wherein said reverse phase chromatography matrix is a polymer resin medium.

20. The process of claim 1, part (l), wherein said washing consists essentially of applying approximately one column volume of 0.2 M acetic acid, containing 0% ethanol; followed by approximately 4 column volumes of 0.2 M acetic acid, containing 19% ethanol.

21. The process of claim 1, part (m), wherein said eluting consists essentially of applying to said reverse phase chromatography matrix approximately four column volumes of 0.2 M acetic acid, containing 19% ethanol, followed by a gradient of 19% to 25% ethanol (v/v), in 0.2 M acetic acid, wherein the gradient is selected from the group consisting of linear gradient and step gradient.

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